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Influence of the CB_1 and CB_2 cannabinoid receptor ligands on the activity of atypical antidepressant drugs in the behavioural tests in mice

Ewa Poleszak[a,*], Sylwia Wośko[a], Karolina Sławińska[a], Elżbieta Wyska[c], Aleksandra Szopa[b], Katarzyna Świąder[a], Andrzej Wróbel[d], Urszula Doboszewska[e], Piotr Wlaź[e], Aleksandra Wlaź[f], Anna Serefko[b,*]

[a] Department of Applied and Social Pharmacy, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland
[b] Department of Applied and Social Pharmacy, Laboratory of Preclinical Testing, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland
[c] Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland
[d] Second Department of Gynecology, Medical University of Lublin, Jaczewskiego 8, 20-090 Lublin, Poland
[e] Department of Animal Physiology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[f] Department of Pathophysiology, Medical University of Lublin, Jaczewskiego 8, 20-090 Lublin, Poland

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ABSTRACT

Available data support the notion that cannabinoids, whose therapeutic value is limited due to severe adverse reactions, could be beneficial as adjunctive agents in the management of mood disorders. Polytherapy, which is superior to monotherapy in the terms of effectiveness, usually requires lower doses of the individual components. Therefore, the main objective of our study was to determine whether administration of cannabinoid (CB) receptor ligands would enhance the antidepressant activity of atypical antidepressant drugs, i.e. agomelatine and tianeptine. To evaluate the antidepressant-like potential of the tested combinations, the mouse forced swim test (FST) and the tail suspension test (TST) were used. The HPLC method was applied to assess the brain levels of agomelatine and tianeptine. Both behavioural tests demonstrated that per se an ineffective intraperitoneal dose of oleamide (CB_1 receptor agonist, 5 mg/kg) potentiated the anti-immobility activity of tianeptine (15 mg/kg), whereas AM251 (CB_1 receptor inverse agonist/antagonist, 0.25 mg/kg) enhanced the antidepressant effects of tianeptine and agomelatine (20 mg/kg). Intraperitoneal co-administration of per se inactive doses of AM630 (CB_2 receptor inverse agonist/antagonist) and agomelatine or tianeptine significantly reduced the immobility time of animals only in the FST. CB receptor ligands did not affect the brain levels of the tested atypical antidepressants. In summary, the outcomes of the present study showed that activation and inhibition of CB_1 receptors as well as inhibition of CB_2 receptors may increase the antidepressant activity of tianeptine, whereas only inhibition of CB_1 and CB_2 receptors has a potential to augment the antidepressant activity of agomelatine.

1. Introduction

Since augmentation and combining therapies have become a common practice in management of the difficult-to-treat depression, new treatment strategies are being searched for. Available data support the notion that adjunctive use of agents modulating different neurochemical pathways involved in depression with antidepressant drugs may alleviate disease symptoms more profoundly than typical antidepressant monotherapy (Ceskova, 2016). Compounds with novel mechanisms of action are particularly under active investigation. Cannabinoids, i.e. ligands of cannabinoid (CB) receptors, belong to these substances. It was confirmed a long time ago that CB receptors play an important role in regulation of the excitatory (glutamate-related) and inhibitory (GABA-related) neurotransmissions in the brain as well as they modulate synthesis and release of monoamines (i.e., dopamine, norephinephrine, serotonin) (Moreira et al., 2009). Literature data also provide reliable evidence that the endocannabinoid system interplays with the hypothalamic–pituitary–adrenal axis, neuroplasticity markers (i.e., the brain-derived neurotrophic factor, BDNF), immune system, or acetylcholine pathways (McLaughlin et al., 2009; Moreira et al., 2009; Zoppi et al., 2014; Kruk-Słomka et al., 2015). All of the above-mentioned signalings are implicated in the pathomechanism of depression. Moreover, under certain conditions, CB receptor ligands display the anxiolytic-like activity (Moreira et al., 2009) and they produce rapid

* Corresponding authors.
E-mail addresses: ewa.poleszak@umlub.pl (E. Poleszak), anna.serefko@umlub.pl (A. Serefko).

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behavioural responses (Linge et al., 2016). Unfortunately, both stimulation and inhibition of the endocannabinoid system (particularly via CB_1 receptors) entail the development of severe adverse reactions (like agitation, aggression, eating disorders, seizures, hypertension, emesis, hypokalaemia) that limit the therapeutic value of CB receptor ligands in monotherapy (Moreira and Crippa, 2009; Hermanns-Clausen et al., 2013). However, CB receptor ligands are still regarded as compounds with a huge therapeutical potential in polytherapy. Polytherapy, which usually requires lower doses of the individual components, could be at least equal to monotherapy in the terms of effectiveness.

Therefore, in the present study we decided to investigate whether CB receptor ligands would enhance the antidepressant activity of atypical antidepressant drugs, i.e. agomelatine and tianeptine. We selected four diverse compounds that interplay differently with CB receptors: (i) oleamide – an agonist of CB_1 receptors, (ii) AM251 – an inverse agonist/antagonist of CB_1 receptors, (iii) JWH133 – an agonist of CB_2 receptors, and (iv) AM630 – an inverse agonist/antagonist of CB_2 receptors. Atypical antidepressants have unique pharmacological properties distinguished by more than one mode of action. In a certain sense, these agents were developed in a response to problems with effectiveness and safety profile of the monoamine based drugs. Biological effects of agomelatine are related to close association between dysfunctions in the circadian rhythms and symptoms of depression, and this multimodal agent agonizes the melatonin M_1 and M_2 receptors as well as it antagonizes the serotoninergic 5-HT_{2B} and 5-HT_{2C} receptors. It also modulates glutamatergic signaling, promotes hippocampal neurogenesis, and reduces neuroinflammation (Guardiola-Lemaitre et al., 2014; Fasipe, 2019). As for tianeptine, the mechanisms of its antidepressant action have not been fully described yet. It is known for sure that this atypical drug neither influences the transporters for noradrenaline or dopamine, nor binds to serotoninergic, dopaminergic, glutamatergic, GABAergic, cholinergic, adrenergic, or histamine receptors. However, it moderately augments the dopamine release via unknown pathways, stabilizes the glutamatergic neurotransmission, increases the level of α1-adrenoceptors, and most probably it potentiates functioning of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors. Additionally, tianeptine acts as an agonist of opioid μ receptors (McEwen et al., 2010).

Scientific justification for carrying out the presented research was based on our previous reports (Poleszak et al., 2019) as well as on the findings of Takahashi et al. (2008) which demonstrated that CB receptor ligands are able to potentiate the activity of common antidepressant drugs that influence the monoaminergic neurotransmission. Takahashi et al. (2008) showed that CB_1 receptor antagonists (SR141716A and AM251) produced an additive effect when administered with selective serotonin reuptake inhibitors (citalopram or sertraline). We gave evidence that both CB_1 receptor stimulation (by oleamide) and inhibition (by AM251) augment the activity of imipramine (a tricyclic antidepressant), escitalopram (a selective serotonin reuptake inhibitor), and reboxetine (a norepinephrine reuptake inhibitor) (Poleszak et al., 2019). Thus, a combination of CB receptor ligands and atypical antidepressant drugs also seemed to us as a promising treatment option. We were also encouraged by the fact that both agomelatine and tianeptine are clinically used as a part of combined therapy with standard antidepressant drugs, and such a polytherapy seems to be a more favourable strategy than monotherapy, particularly in the treatment-resistant cases (Tobe and Rybakowski, 2013; Fasipe, 2019; Potměšil, 2019).

2. Materials and methods

The experiments were carried out in accordance with binding law related to studies on animal models as well as in compliance with the protocol approved by the Local Ethics Committee.

2.1. Animals

Drug and test naïve adult male Albino Swiss mice (8–10 weeks old, 25–30 g) provided by the Centre for Experimental Medicine (OMD) at the Medical University of Lublin were used in the study. Animals were kept in standard cages (8 individuals/cage) in the environmentally controlled facility, i.e. 12 h day/night cycle, temperature of 22–23 °C, humidity of 45–55%, with free access to both water and food. The bedding was corncob granules and it was changed once a week. The refinement principle minimizing potential distress and enhancing animal welfare was applied. Overall 356 mice were randomly assigned to experimental groups. Each testing group was represented by 7–10 animals, depending on the research schedule. All behavioural experiments were performed between 8:00 and 15:00.

2.2. Drugs

The following agents were tested in the study: (i) CB_1 receptor ligands – oleamide (*cis*-9,10-octadecenoamide, Tocris, USA) and AM251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, Tocris, USA), (ii) CB_2 receptor ligands – JWH133 ((6aR,10aR)-3-(1,1-Dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[*b*,*d*]pyran, Tocris, USA) and AM630 (6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone, Tocris, USA), and (iii) atypical antidepressants – agomelatine (Sigma-Aldrich, USA) and tianeptine (Sigma-Aldrich, USA). The CB receptor ligands and agomelatine were suspended in an aqueous solution of Tween 80 (1%), whereas tianeptine was dissolved in saline. Prepared suspensions and the solution were given as intraperitoneal (*ip*) injections: agomelatine and tianeptine – 60 min before testing, and oleamide, AM251, JWH133, and AM630 – 30 min before testing. The control animals received *ip* injections of vehicles, i.e. saline and the aqueous solution of Tween 80 (1%). The tested doses as well as the pretreatment schedules were chosen on the basis of the results of our previous projects (e.g., Szopa et al., 2019) and the literature data (Kruk-Słomka et al., 2015).

The following experimental groups were tested:

(i) the control group that received vehicles, i.e. saline + aqueous solution of Tween 80 (1%)
(ii) animals that received oleamide (5 mg/kg) + saline
(iii) animals that received AM251 (0.25 mg/kg) + saline
(iv) animals that received JWH133 (0.25 mg/kg) + saline
(v) animals that received AM630 (0.25 mg/kg) + saline
(vi) animals that received agomelatine (20 mg/kg) + saline
(vii) animals that received tianeptine (15 mg/kg) + aqueous solution of Tween 80 (1%)
(viii) animals that received oleamide (5 mg/kg) + agomelatine (20 mg/kg)
(ix) animals that received oleamide (5 mg/kg) + tianeptine (15 mg/kg)
(x) animals that received AM251 (0.25 mg/kg) + agomelatine (20 mg/kg)
(xi) animals that received AM251 (0.25 mg/kg) + tianeptine (15 mg/kg)
(xii) animals that received JWH133 (0.25 mg/kg) + agomelatine (20 mg/kg)
(xiii) animals that received JWH133 (0.25 mg/kg) + tianeptine (15 mg/kg)
(xiv) animals that received AM630 (0.25 mg/kg) + agomelatine (20 mg/kg)
(xv) animals that received AM630 (0.25 mg/kg) + tianeptine (15 mg/kg)

2.3. Forced swim test (FST)

The FST was carried out according to the procedure described by Porsolt et al. (1977). Briefly, each mouse was placed individually into a glass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C. An animal was left there for 6 min. Immobility of a mouse, i.e. duration of time when a given animal stopped struggling in the water and performed only movements necessary to keep its head over the water surface, was measured between the 2nd and the 6th min of the test.

2.4. Tail suspension test (TST)

The TST was carried out according to the procedure described by Steru et al. (1985). Each mouse was suspended by the tail (2 cm from the end of the tail) about 50 cm above the floor, using an adhesive tape. It was left there for 6 min. Immobility of animals, i.e. duration of time when a suspended mouse stopped struggling in the air and performed only movements necessary to breathe, was measured between the 2nd and the 6th min of the test.

2.5. Spontaneous locomotor activity

Measurements of spontaneous locomotor activity was carried out according to the procedure we had used before (Szopa et al., 2019). An activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, USA) was used. The device consists of 4 transparent cages with lids equipped with 4 infrared emitters with laser beams and 4 detectors monitoring animal movements. Each mouse was placed individually into the cage (43 cm × 43 cm × 32 cm) and left there for 6 min. A distance travelled by a given animal was recorded automatically during the time interval corresponding to the one analyzed in the FST and the TST, that is between the 2nd and the 6th min of the test.

2.6. Determination of the brain levels of agomelatine and tianeptine

60 min following injection of a given atypical antidepressant (with or without a CB receptor ligand), the tested animals were decapitated and their brains were collected and frozen. The brain levels of agomelatine and tianeptine were assessed by the high-performance liquid chromatography (HPLC) method, according to the procedure we had used before (Szopa et al., 2019). The brains were homogenized in distilled water (1:4, w/v) with a TH220 tissue homogenizer (Omni International, Inc., Warrenton, VA, USA). For agomelatine, 1 ml of brain homogenate was spiked with carbamazepine (100 ng/ml) as an internal standard (IS). Before the extraction, 1 ml of the concentrated NaCl solution (10 g/50 ml) was added to brain homogenate and the samples were vortexed for 15 s. The extraction of agomelatine from brain homogenate was performed using 5 ml of a mixture of dichloromethane/hexane/isoamyl alcohol (39.5:59.5:1 v/v/v). The samples were shaken for 20 min and centrifuged for 15 min at 1000 ×*g*. After the centrifugation, the organic layers were transferred into conical glass tubes and evaporated to dryness at 37 °C under a gentle stream of nitrogen in a water bath. The residues were dissolved with 100 µl of methanol, and aliquots of 50 µl were injected into the HPLC system. Carbamazepine (250 ng/ml) was also used as IS for tianeptine. 10 µl of this solution was added to the brain homogenate (1 ml) containing tianeptine. 1 ml of phosphate buffer (0.5 M, pH 7.0) was given to the samples. Next, the samples were extracted with 3 ml of the extraction reagent, i.e. a mixture of ethyl acetate/hexane (30:70, v/v), by shaking for 20 min (IKA Vibrax VXR, Germany). After centrifugation at 3000 rpm for 15 min (Universal 32, Hettich, Germany), the organic layers were transferred into conical glass tubes and evaporated to dryness at 37 °C under a gentle stream of nitrogen in a water bath. The residues were dissolved with 100 µl of methanol, and aliquots of 50 µl were injected into the HPLC system.

The HPLC system consisted of an isocratic pump (model L-7100) and an autosampler (model L-7200), both from Merck Hitachi (Darmstadt, Germany), and a UV variable-wavelength K-2600 detector (Knauer, Berlin, Germany). Data acquisition and processing were carried out using the D-7000 HSM software (Merck Hitachi). Analysis of agomelatine and tianeptine was performed on a 250 × 4 mm LiChrospher1100 RP-18 column with a particle size of 5 µm (Merck, Darmstadt, Germany) protected with a guard column (4 × 4 mm) with the same packing material. The mobile phase consisting of acetonitrile and 50 mM potassium dihydrogen phosphate was mixed at a ratio of 37:63 (v/v) for agomelatine and 31:69 (v/v) for tianeptine and run at 1 ml/min. Chromatographic analysis was carried out at 21 °C and an analytical wavelength of 230 nm for agomelatine and 214 nm for tianeptine.

The calibration curves constructed by plotting the ratio of the peak heights of the studied drug to IS versus the concentration of the drug were linear in the tested concentration ranges. No interfering peaks were observed in the chromatograms. The measurements were reproducible with low intra- and interday variation (coefficient of variation < 10%). The extraction efficiencies of the analyzed compounds and the internal standard ranged from 70% to 95%. Levels of the tested antidepressants were given in ng/g (for the wet brain tissue).

2.7. Statistical analysis

Statistical analysis was performed either by two-way analysis of variance (ANOVA) with Bonferroni's *post-hoc* test or by *t*-test, depending on the study design. The results from the behavioural tests were calculated by two-way ANOVA, whereas the outcomes from the pharmacokinetic analyses were calculated by *t*-test. In two-way ANOVA, the following independent variables were taken into consideration: (i) treatment with an atypical antidepressant, and (ii) treatment with a CB receptor ligand. The results were presented as the means ± standard error of the mean (SEM). Between-group differences with p lower than 0.05 were treated as statistically significant (where: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$).

3. Results

An acute *ip* injection of oleamide (5 mg/kg), AM251 (0.25 mg/kg), JWH133 (0.25 mg/kg), AM630 (0.25 mg/kg), agomelatine (20 mg/kg), or tianeptine (15 mg/kg) did not change the immobility time of the tested mice in the FST and in the TST, when compared to the vehicle-treated group.

3.1. Effects of a concomitant administration of oleamide and the atypical antidepressants in the FST and the TST

As presented in Fig. 1, an acute injection of oleamide (5 mg/kg) did not potentiate the activity of agomelatine given at a dose of 20 mg/kg either in the FST or in the TST. Though Bonferroni's *post-hoc* test detected significant differences in the FST between the group that received oleamide + agomelatine and the group that received only agomelatine, two-way ANOVA demonstrated a non-significant oleamide-agomelatine interaction in the FST [$F(1,35) = 1.07$; $p = 0.3074$] and also a non-significant oleamide-agomelatine interaction in the TST [$F(1,36) = 1.03$; $p = 0.3178$]. On the other hand, oleamide managed to augment the anti-immobility effect of tianeptine (15 mg/kg) in both applied tests. Statistical analysis by two-way ANOVA confirmed a significant oleamide-tianeptine interaction in the FST [$F(1,34) = 5.68$; $p = 0.0229$] with a significant effect of oleamide [$F(1,34) = 18.72$; $p = 0.0001$] and a significant effect of tianeptine [$F(1,34) = 6.01$; $p = 0.0195$]. Similarly, two-way ANOVA demonstrated a significant oleamide-tianeptine interaction in the TST [$F(1,36) = 17.43$; $p = 0.0002$] with a significant effect of oleamide [$F(1,36) = 10.71$; $p = 0.0024$] and a significant effect of tianeptine [$F(1,36) = 12.21$;

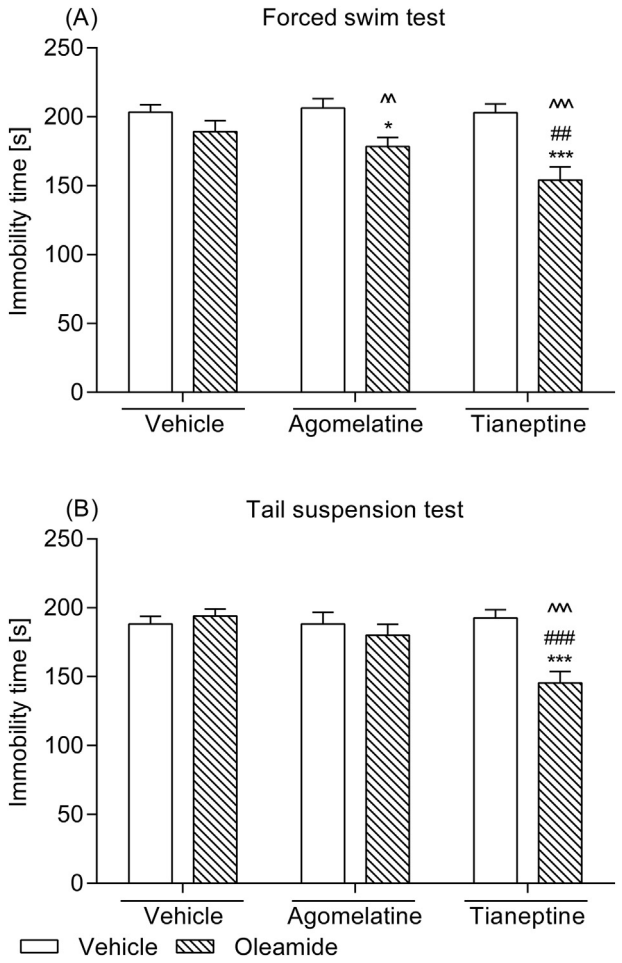

Fig. 1. Effect of a concurrent intraperitoneal administration of oleamide and atypical antidepressants in (A) the FST and (B) the TST in mice.
Oleamide (5 mg/kg) was given 30 min before the experiment, while agomelatine (20 mg/kg) and tianeptine (15 mg/kg) were injected 60 min before testing. The values represent mean + SEM (n = 9–10 animals per group). ^^p < 0.01, ^^^p < 0.01 versus respective antidepressant drug; $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 versus oleamide; *p < 0.05, ***p < 0.001 versus vehicle (two-way ANOVA followed by Bonferroni's *post-hoc* test).

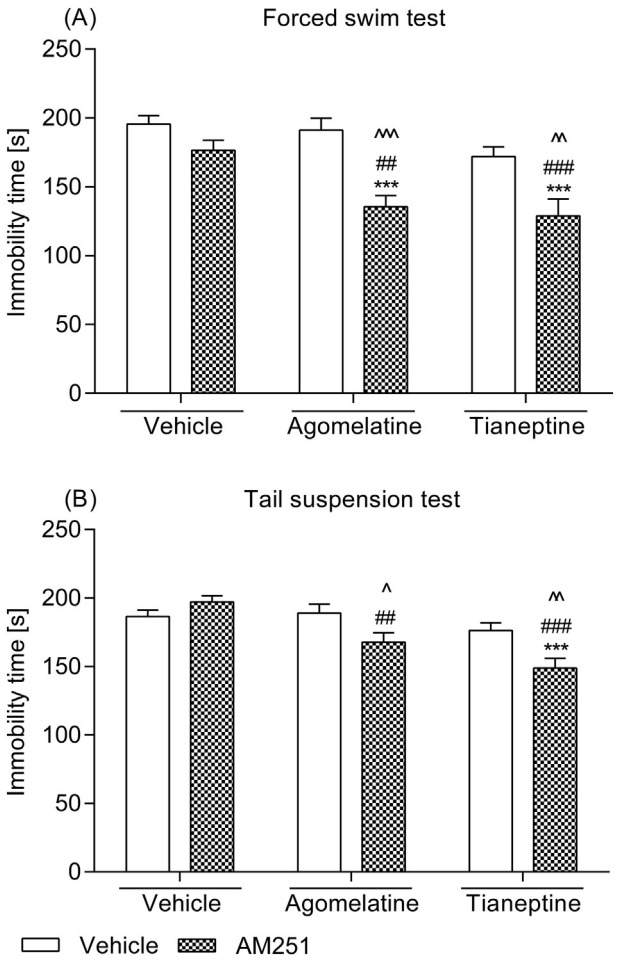

Fig. 2. Effect of a concurrent intraperitoneal administration of AM251 and atypical antidepressants in (A) the FST and (B) the TST in mice.
AM251 (0.25 mg/kg) was given 30 min before the experiment, while agomelatine (20 mg/kg) and tianeptine (15 mg/kg) were injected 60 min before testing. The values represent mean + SEM (n = 7–8 animals per group). ^p < 0.05, ^^p < 0.01, ^^^p < 0.01 versus respective antidepressant drug; $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001 versus AM251; ***p < 0.001 versus vehicle (two-way ANOVA followed by Bonferroni's *post-hoc* test).

p = 0.0013].

3.2. Effects of a concomitant administration of AM251 and the atypical antidepressants in the FST and the TST

After combined administration of AM251 (0.25 mg/kg) and agomelatine (20 mg/kg) or tianeptine (15 mg/kg), the tested mice struggled for a longer time when placed in the water or suspended by their tails in comparison to the animals that were given the respective monotherapy (Fig. 2). Two-way ANOVA revealed: (1) a significant AM251-agomelatine interaction [F(1,28) = 5.61; p = 0.0250] in the FST, with a significant effect of AM251 [F(1,28) = 23.29; p < 0.0001] and a significant effect of agomelatine [F(1,28) = 8.61; p = 0.0066], (2) a significant AM251-agomelatine interaction [F(1,26) = 8.23; p = 0.0081] in the TST, with a significant effect of agomelatine [F(1,26) = 5.75; p = 0.0240] but a not significant effect of AM251 [F(1,26) = 0.91; p = 0.3499], (3) a significant AM251-tianeptine interaction [F(1,26) = 4.33; p = 0.0475] in the FST, with a significant effect of AM251 [F(1,26) = 9.06; p = 0.0057] and a significant effect of tianeptine [F(1,26) = 20.64; p = 0.0001], (4) a significant AM251-tianeptine interaction [F(1,28) = 11.40; p = 0.0022] in the TST, with a significant effect of tianeptine [F(1,28) = 26.95; p < 0.0001] but a not significant effect of AM251 [F(1,28) = 2.17; p = 0.1521].

3.3. Effects of a concomitant administration of JWH133 and the atypical antidepressants in the FST and the TST

Addition of JWH133 (0.25 mg/kg) to the treatment with agomelatine (20 mg/kg) or tianeptine (15 mg/kg) did not potentiate the activity of the tested atypical antidepressants in either of the applied tests. The animals that received JWH133 + agomelatine or JWH133 + tianeptine behaved almost in the same manner in the FST and in the TST as the mice that received only JWH133 or the respective antidepressant drug. Though Bonferroni's *post-hoc* test detected significant differences in the FST between the group that received JWH133 + tianeptine and the groups that received only JWH133 or the vehicle, two-way ANOVA did not detect any significant drug-drug interaction for the JWH133-tianeptine treatment in the FST (F(1,28) = 1.03; p = 0.3182) or in the TST (F(1,28) = 1.02; p = 0.3211). Similarly, a non-significant interaction was obtained for the JWH133-agomelatine treatment in the FST (F(1,28) = 2.73; p = 0.1094) and in the TST (F(1,28) = 0.08; p = 0.7846). The results were illustrated in Fig. 3.

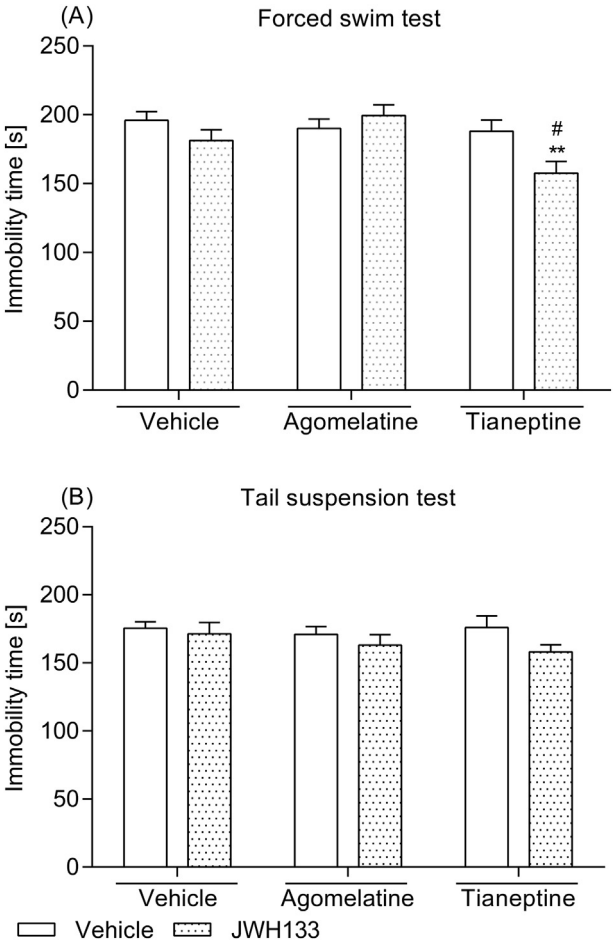

Fig. 3. Effect of a concurrent intraperitoneal administration of JWH133 and atypical antidepressants in (A) the FST and (B) the TST in mice.
JWH133 (0.25 mg/kg) was given 30 min before the experiment, while agomelatine (20 mg/kg) and tianeptine (15 mg/kg) were injected 60 min before testing. The values represent mean + SEM (n = 8 animals per group). $^{\#}p < 0.05$ versus JWH133; $^{**}p < 0.01$ versus vehicle (two-way ANOVA followed by Bonferroni's *post-hoc* test).

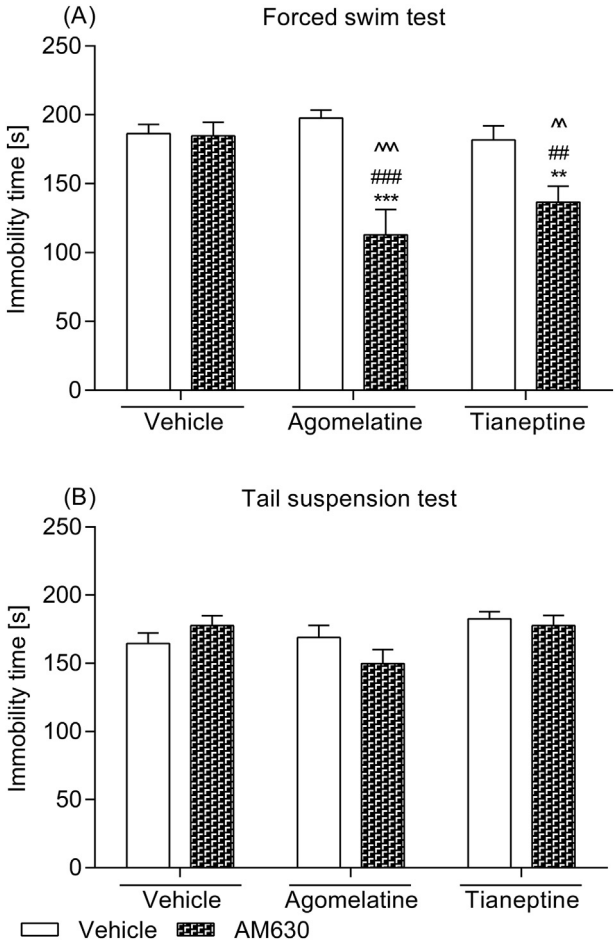

Fig. 4. Effect of a concurrent intraperitoneal administration of AM630 and atypical antidepressants in (A) the FST and (B) the TST in mice.
AM630 (0.25 mg/kg) was given 30 min before the experiment, while agomelatine (20 mg/kg) and tianeptine (15 mg/kg) were injected 60 min before testing. The values represent mean + SEM (n = 7–8 animals per group). $^{\wedge}p < 0.01$, $^{\wedge\wedge}p < 0.01$ versus respective antidepressant drug; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus AM630; $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle (two-way ANOVA followed by Bonferroni's *post-hoc* test).

3.4. Effects of a concomitant administration of AM630 and the atypical antidepressants in the FST and the TST

Mice that received a combination of AM630 (0.25 mg/kg) and agomelatine (20 mg/kg) or tianeptine (15 mg/kg) were actively moving for a longer time in the FST in comparison to animals from the control groups. Such an effect was not observed in the TST – mice treated with a given concomitant therapy stayed immobile for a similar duration of time as the animals subjected to the respective monotherapy (Fig. 4). Calculations with two-way ANOVA showed a significant AM630-agomelatine interaction [$F(1,28) = 13.26$; $p = 0.0011$] in the FST, with a significant effect of AM630 [$F(1,28) = 14.32$; $p = 0.0007$] and a significant effect of agomelatine [$F(1,28) = 7.05$; $p = 0.0129$], but a not significant AM630-agomelatine interaction [$F(1,28) = 3.45$; $p = 0.0736$] in the TST. Correspondingly, according to the statistical outcomes, AM630-tianeptine interaction in the FST was significant [$F(1,27) = 4.98$; $p = 0.0341$], with a significant effect of both AM630 [$F(1,27) = 5.75$; $p = 0.0237$] and tianeptine [$F(1,27) = 7.19$; $p = 0.0124$], but it was not significant in the TST [$F(1,28) = 1.67$; $p = 0.2071$].

3.5. Effects of a concomitant administration of the CB receptor ligands and the atypical antidepressants on the spontaneous locomotor activity of mice

None of the tested agents (i.e., oleamide, AM251, JWH133, AM630, agomelatine, and tianeptine) or their respective combinations significantly increased the spontaneous locomotor activity of mice (Table 1).

3.6. Brain levels of agomelatine and tianeptine

In the statistical analysis of outcomes from the pharmacokinetic assay, we took into consideration only these combinations that had acted synergistically in the behavioural tests, i.e. AM251 (0.25 mg/kg) or AM630 (0.25 mg/kg) with agomelatine (20 mg/kg), and oleamide (5 mg/kg), AM251, or AM630 with tianeptine (15 mg/kg). As presented in Table 2, none of the tested CB receptor ligands increased or reduced the brain levels of the atypical antidepressants. Calculations with *t*-test gave the following results: (1) $t(14) = 0.1431$, $p = 0.8882$ for the AM251-agomelatine combination, (2) $t(13) = 1.895$, $p = 0.0806$ for the AM630-agomelatine combination, (3) $t(18) = 0.4003$, $p = 0.6937$ for the oleamide-tianeptine combination, (4) $t(14) = 1.104$, $p = 0.2882$ for the AM251-tianeptine combination, and (5) $t(14) = 0.8493$, $p = 0.4100$ for the AM630-tianeptine combination.

Table 1
Effect of a combined intraperitoneal administration of (A) oleamide, (B) AM251, (C) JWH133, or (D) AM630 and atypical antidepressant drugs on the spontaneous locomotor activity of mice.

	Treatment (n = number of mice per group)	Travelled distance (cm)
(A)	Vehicle + vehicle (n = 8)	662.6 ± 42.78
	Oleamide + vehicle (n = 8)	511.5 ± 83.66
	Agomelatine + vehicle (n = 8)	530.1 ± 83.95
	Agomelatine + oleamide (n = 7)	420.9 ± 59.87
	Tianeptine + vehicle (n = 8)	594.8 ± 77.18
	Tianeptine + oleamide (n = 7)	382.0 ± 80.17
(B)	Vehicle + vehicle (n = 8)	629.0 ± 82.22
	AM251 + vehicle (n = 8)	647.6 ± 47.97
	Agomelatine + vehicle (n = 8)	674.9 ± 37.73
	Agomelatine + AM251 (n = 8)	560.6 ± 32.65
	Tianeptine + vehicle (n = 8)	720.6 ± 130.3
	Tianeptine + AM251 (n = 8)	856.9 ± 74.32
(C)	Vehicle + vehicle (n = 8)	585.6 ± 49.79
	JWH133 + vehicle (n = 8)	567.0 ± 53.30
	Agomelatine + vehicle (n = 8)	561.5 ± 48.07
	Agomelatine + JWH133 (n = 8)	555.9 ± 40.16
	Tianeptine + vehicle (n = 8)	565.6 ± 103.5
	Tianeptine + JWH133 (n = 8)	561.3 ± 72.00
(D)	Vehicle + vehicle (n = 8)	402.0 ± 82.89
	AM630 + vehicle (n = 8)	544.9 ± 34.57
	Agomelatine + vehicle (n = 8)	407.8 ± 73.66
	Agomelatine + AM630 (n = 8)	545.9 ± 65.85
	Tianeptine + vehicle (n = 8)	536.6 ± 101.4
	Tianeptine + AM630 (n = 8)	681.5 ± 138.9

Oleamide (5 mg/kg), AM251 (0.25 mg/kg), JWH133 (0.25 mg/kg), and AM630 (0.25 mg/kg) were given 30 min before the experiment, while agomelatine (20 mg/kg) and tianeptine (15 mg/kg) were injected 60 min before testing. The values represent mean ± SEM (two-way ANOVA followed by Bonferroni's *post-hoc* test).

Table 2
Effects of CB receptor ligands on the brain levels of atypical antidepressants in mice.

Treatment	Drug level in the brain (ng/g)	Number of animals per group
Agomelatine + vehicle	9.204 ± 2.080	8
Agomelatine + AM251	8.850 ± 1.336	8
Agomelatine + vehicle	13.77 ± 3.859	7
Agomelatine + AM630	6.056 ± 1.785	8
Tianeptine + vehicle	37.83 ± 5.605	10
Tianeptine + oleamide	35.14 ± 3.735	10
Tianeptine + vehicle	10.18 ± 0.9068	8
Tianeptine + AM251	14.74 ± 4.033	8
Tianeptine + vehicle	16.32 ± 2.566	8
Tianeptine + AM630	12.15 ± 4.186	8

Oleamide (5 mg/kg), AM251 (0.25 mg/kg), and AM630 (0.25 mg/kg) were administered intraperitoneally 30 min before decapitation, whereas tianeptine (15 mg/kg) and agomelatine (20 mg/kg) were injected intraperitoneally 60 min before decapitation. The values represent mean ± SEM (*t*-test).

4. Discussion

To the best of our knowledge this is the first report of a positive interaction between CB_1 and CB_2 receptor ligands and atypical antidepressant drugs in the FST and the TST in mice. Activity of tianeptine (15 mg/kg) was potentiated by opposing pharmacological interventions towards CB_1 receptors, i.e. by administration of per se ineffective doses of their agonist (oleamide, 5 mg/kg) and inverse agonist/antagonist (AM251, 0.25 mg/kg). We demonstrated that the antidepressant effect of tianeptine was also augmented by per se an inactive dose of the inverse agonist/antagonist of CB_2 receptors (AM630, 0.25 mg/kg), whereas it was not affected by the CB_2 receptor agonist (JWH133, 0.25 mg/kg). As for the activity of agomelatine, it was enhanced only by inhibition of either CB_1 or CB_2 receptor functioning. Stimulation of CB_1 or CB_2 receptors had no impact on the antidepressant potential of this drug. We should also underline that positive interactions between the CB_1 or CB_2 receptor antagonists and the tested antidepressants were definitely more pronounced in the FST than in the TST. Differences in the sensitivity of the tests were particularly noticed in the case of AM630, since only the FST detected significant AM630-agomelatine and AM630-tianeptine interactions. According to outcomes obtained in the TST, these substances did not mutually potentiate each other's activity when given in the respective combinations. Following Cryan et al. (2005), the FST and the TST may respond differently to a given drug, and antidepressant-like effects of some atypical agents can be detected in the swimming test but not seen in the suspension test, as it happened in our study. Despite several reports that cannabinoids may change the spontaneous motility of rodents (Onaivi et al., 2008; Kruk-Słomka et al., 2015), neither hyperkinesia nor hypolocomotion confounded our results in the FST and the TST. Animals from all tested groups travelled the same distance as their vehicle-treated counterparts. Furthermore, the introduced treatment was well tolerated by the mice since they did not manifest any visible signs of health deterioration.

The outcomes of our experiments are partially in line with the well-known bi-directional activity of the CB_1 receptor-related signaling. Generally, the endocannabinoid system and its regulatory mechanisms appear to be highly complicated and complex. On the one hand, the dampened endocannabinoid-dependent signaling is related to the depression-like phenotype (Smaga et al., 2017) and its stimulation via CB_1 receptors activation, inhibition of fatty acid amide hydrolase (i.e., an endogenous enzyme that breaks down anandamide), or via reduction of endocannabinoid uptake has an antidepressant-like potential (Adamczyk et al., 2008; Bambico et al., 2010). On the other hand, inverse agonists/antagonists of CB_1 receptors (such as AM251 or rimonabant) also exert anti-immobility effects in the FST (Shearman et al., 2003; Griebel et al., 2005; Kruk-Słomka et al., 2015) and the TST (Shearman et al., 2003; Khakpai et al., 2019) in rodents. Similarly, both CB_2 receptor agonists (e.g. JWH133) and inverse agonists/antagonists (e.g. AM630) have displayed the antidepressant-like activity in the recognized behavioural tests (García-Gutiérrez et al., 2010; Kruk-Słomka et al., 2015; Khakpai et al., 2019). Furthermore, there are reports of the pro-depressive effects induced by the enhancement of the endocannbinoid signaling (Pertwee, 2015). The dual action of the endocannabinoid system has been detected for the anxiolytic-like/anxiogenic effects, as well. In fact, it is difficult to say why agonists and antagonists of CB_1 and CB_2 receptors can have the same activity in the FST and the TST. Some authors (Häring et al., 2013) assume that the specific localization of CB receptors could be one of the reasons for the contradictory outcomes from the pre-clinical studies focused on the role of the endocannabinoid system in the pathogenesis of anxiety and depression. CB_1 receptors are localized in GABA-ergic (i.e., inhibitory) and glutamatergic (i.e., excitatory) terminals, thus they have at the same time an impact on the opposing neurotransmissions. Other authors suggest that several other factors may contribute to the final outcomes, such as: (1) an animal model/strain, their baseline stress level, experimental conditions, or an applied dose of a given cannabinoid (e.g., Griebel et al., 2005; Bambico et al., 2010; Beyer et al., 2010), (2) existence of the functional pools of receptors that are involved in manifestation of the depressogenic and antidepressant effects (Patel and Hillard, 2009), and (3) the existence of some unknown subtypes of endocannabinoid receptors (Ostadhadi et al., 2016).

Literature data clearly indicate that CB_1 receptors directly and indirectly modulate the monoaminergic system. They are present on both serotonin and norepinephrine neurons in diverse parts of the brain as well as they can be found in limbic mood-regulatory areas rich in dopamine. It has been demonstrated that CB_1 receptor ligands influence the firing of monoaminergic neurons and have an impact on the dopa/dopamine, norepinephrine, and serotonin synthesis as well as on dopamine, norepinephrine, serotonin, glutamate, and GABA release in specific brain regions (Esteban and García-Sevilla, 2012). As for CB_2

receptor ligands, modulation of the serotonin-, glutamate-, immune-, and/or neurotrophin-related pathways may be involved in their antidepressant-like activity (Benito et al., 2008; García-Gutiérrez et al., 2010; Zoppi et al., 2014; Ishiguro et al., 2018). Therefore, we assume that the enhancement of the serotoninergic neurotransmission may be particularly responsible for the observed interactions between CB receptor ligands and agomelatine, whereas potentiation of the dopaminergic signaling may be mainly responsible for the positive interplay between CB receptor ligands and tianeptine. Keeping in mind that AM630 is not only an inverse agonist/antagonist of CB_2 receptors but it also has an affinity towards CB_1 receptors and it acts as their inverse agonist (Landsman et al., 1998), one can suspect that an interplay between AM630 and CB_1 receptors can contribute to the final effects of AM630 treatment in our studies. We guess that co-administration of oleamide and agomelatine as well as concurrent use of JWH133 and agomelatine or tianeptine were unable to sufficiently increase the levels of monoamines, and this is the reason why these combinations did not induce shortening of the immobility time of animals in the applied behavioural tests. Of note is the fact that the antidepressant activity of agomelatine is partially due to the inhibition of serotoninergic 5-HT_{2A} receptors, whereas in studies by Franklin et al. (2013) JWH133 has emerged as an agent that upregulates these receptors. So, the opposite activity towards serotoninergic 5-HT_{2A} receptors may be responsible for the lack of the antidepressant potential of the JWH133-agomelatine combination.

Drug-drug interactions detected in our study are most probably due to mechanisms that take place at the cellular level, since our pharmacokinetic studies did not reveal significant alterations in the brain levels of the tested atypical antidepressants after concomitant administration with CB receptor ligands. Based on the outcomes of Smaga et al. (2017), an acute administration of tianeptine could have increased CB_1 density in different parts of the brain, including the motor cortex, frontal cortex, and hippocampus. However, we should not expect an intensified expression of CB_1 and CB_2 receptors in the hippocampus, frontal cortex and/or prefrontal cortex – such effects were detected only after chronic, not acute, treatment with tianeptine (10 mg/kg).

We think that the main limitation of the current study is lack of experiments assessing the brain levels of neurotransmitters involved in the development of depression. In fact, we are going to carry out such analyses in future projects, since they would enable better understanding of the molecular mechanisms implicated in the interplay between the endocannabinoid system and antidepressant drugs. Moreover, without running an isobologram analysis we are not able to conclude whether the drug combinations used in our studies produced additive or synergistic effects.

5. Conclusions

To the best of our knowledge this is the first report of a positive interaction between CB_1 and CB_2 receptor ligands and atypical antidepressant drugs in the FST and the TST in mice. Accordingly, the following outcomes of the presented experiments should be particularly emphasized: (1) both activation and inhibition of CB_1 receptors as well as inhibition of CB_2 receptors may increase the antidepressant activity of tianeptine, (2) inhibition of CB_1 and CB_2 receptors has a potential to augment the antidepressant activity of agomelatine, (3) the observed interactions have rather the pharmacodynamic background instead of the pharmacokinetic one.

These preliminary findings need to be confirmed in further experiments, but it seems that the adjuvant therapy to atypical antidepressants based on modulation of the endocannabinoid system (particularly via CB_1 receptors) could be a promising treatment option. Such a strategy may be beneficial in patients suffering from depression that co-exists with anxiety, since CB receptor ligands, agomelatine, and tianeptine have a certain anxiolytic potential (Pertwee, 2015; Brink et al., 2006; McEwen et al., 2010; Fasipe, 2019).

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